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## Alzheimer's disease from phenotype to genotype

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# Chapter 3.1

## ***SORL1* variant co-occurs with *APOE*- $\epsilon$ 4 homozygosity in an Alzheimer's disease family**

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Submitted

**ABSTRACT**

**Objective** We aimed to detect the underlying genetic cause of Alzheimer's disease (AD) in a family with heterogeneous clinical symptoms.

**Methods** DNA from three family members affected with (preclinical) AD, and one healthy relative was analyzed with whole exome sequencing. Sanger sequencing was used to confirm the detected variants, and the segregation patterns in one affected and six unaffected additional family members. *APOE* genotype was available for all participants.

**Results** All affected family members presented with a relatively late age of clinical symptom onset, but the presence of microbleeds/cerebral amyloid angiopathy (CAA) and electroencephalographic abnormalities differed for each individual. We detected a rare missense variant in the Sortilin-related receptor gene (*SORL1*), p.N674S, in combination with *APOE*- $\epsilon$ 4 homozygosity in all four affected family members with (preclinical) AD, and in one younger family member who currently has no signs of cognitive decline. Three healthy relatives carried the *SORL1* variant but were heterozygous for the *APOE*- $\epsilon$ 4 allele. We did not detect the rare *SORL1* variant in a larger case-control dataset.

**Conclusion** We describe an AD family presenting a rare *SORL1* variant co-occurring with *APOE*- $\epsilon$ 4 homozygosity. We hypothesize a combined effect of both risk variants on disease pathology.

## INTRODUCTION

Alzheimer's disease (AD) is a complex and heterogeneous neurodegenerative disease. Less than 1% of AD cases are caused by an autosomal dominant mutation, with a typical age at onset before the age of 60 years.<sup>1</sup> Mutations associated with autosomal dominant AD have been detected in three genes; the amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*).<sup>2-4</sup> However, a causative mutation in one of the three genes can be detected in only 50% of the AD patients with a clear autosomal dominant inheritance pattern,<sup>1</sup> suggesting the presence of other causative genes.

Genetic factors are estimated to account for 60-80% of late-onset AD (LOAD) risk,<sup>5</sup> and 10-20% is accounted for by Apolipoprotein-ε4 (*APOE*) genotype.<sup>6</sup> *APOE*-ε4 predisposes for AD with a typical amnesic phenotype, yet with an earlier age of disease onset,<sup>7</sup> and an increased risk of cerebral amyloid angiopathy (CAA).<sup>8</sup> Many other genes have been found to be associated with the incidence of LOAD, although with only small effect sizes.<sup>9</sup> One of these genes is the Sortilin-related receptor gene (*SORL1*).<sup>10,11</sup> Moreover, *SORL1* variants have also been associated with autosomal dominant AD.<sup>12</sup> We present a rare missense variant in *SORL1* in combination with *APOE*-ε4 homozygosity, detected in a Dutch family showing an autosomal dominant inheritance pattern of relatively late onset AD with clinical heterogeneity.

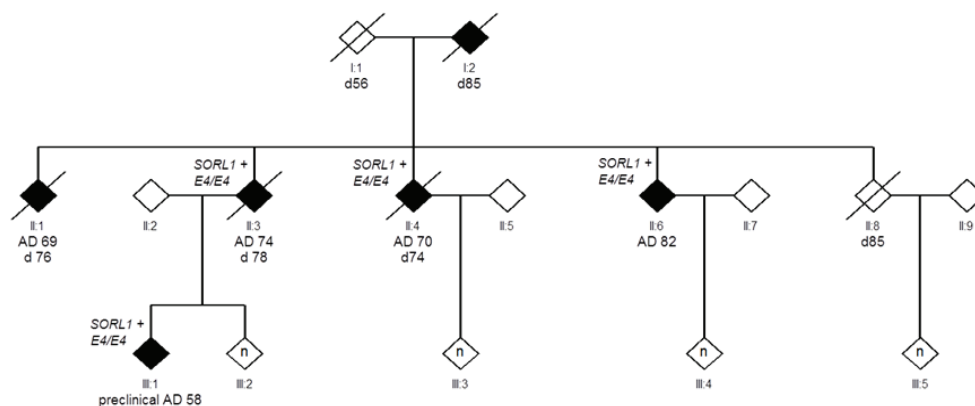
## METHODS

### Participants

We describe six family members diagnosed with (preclinical) AD, who span three generations within one pedigree (figure 1). Clinical data were not available for the affected family members of the first generation (I.2). Three family members, II.1, II.6, and III.1, visited the Alzheimer center at the VU University medical center (VUmc) and underwent extensive standardized diagnostic work-up.<sup>13</sup> The other family members were diagnosed elsewhere in the Netherlands. Post-mortem autopsy results of individual II.4 were revised by our neuropathologist.

The local medical ethics committee of the VUmc approved the study, and all participating family members or their representatives gave written informed consent.

Figure 1. Pedigree of the described family



Sex is not indicated to avoid recognition.

*SORL1* + indicates that the subject is positive for the variant c.2012A>G in *SORL1*. E4/E4 indicates APOE-ε4 homozygosity. 'n' in a diamond indicates more than one individual. Consequently, the ID under the symbols with an 'n' refers to multiple family members as a group.

AD [number]: Alzheimer's disease with age at diagnosis. d [number]: age at death

## DNA analysis

DNA was isolated from peripheral blood from four family members. The exomes were captured by the Nimblegen human exome v3 capture kit, and 2x100 paired-end sequencing reads were generated on the Illumina HiSeq 2000 platform, according to the manufacturer's protocol. Reads were mapped to the human reference genome sequence (UCSC hg19) using the Burrows-Wheeler alignment tool.<sup>14</sup> Duplicate read removal, local sequence realignment and base quality recalibration were performed by Picard (<http://picard.sourceforge.net>) and with GATK (genome analysis tool kit).<sup>15</sup> Variants were called using GATK haplotype caller, and filtered using the variant filtration tool. For each variant we set the filter to PASS if the variant complied with (I) GATK quality score  $\geq 50$ , (II) quality over depth  $\geq 1.5$ , (III) Strand bias  $\leq 60$ , (IV) total read depth  $\geq 5.0$ . Variants were annotated and analyzed with Cartagenia (<http://www.cartagenia.com/>) using a filter tree specifically designed to detect variants causative for a trait with an autosomal dominant inheritance pattern. Variants were selected for further analysis if (I) absent in the following databases: dbSNP138 ([http://www.ncbi.nlm.nih.gov/projects/SNP/build\\_138](http://www.ncbi.nlm.nih.gov/projects/SNP/build_138)), the 1.000 genome project ([www.1000genomes.org](http://www.1000genomes.org)) or the National heart lung blood institute exome variant server (EVS) (<http://evs.gs.washington.edu/EVS/>); (II) a prevalence of  $\leq 5\%$  in the whole Alzheimer cohort; (III) heterozygote in all 3 affected family members; (IV) listed in the OMIM database ([www.omim.org](http://www.omim.org)). The predicted functional effects of the selected sequence variants were assessed by PolyPhen2 ([genetics.bwh.harvard.edu/pph2/](http://genetics.bwh.harvard.edu/pph2/)), SIFT ([sift.jcvi.org/](http://sift.jcvi.org/)), Mutation Taster ([www.mutationtaster.org](http://www.mutationtaster.org)), and the combined annotation dependent deletion (CADD)

score.<sup>16</sup> Information about localization and conservation of the selected variants was assessed by Uniprot ([www.uniprot.org/uniprot/Q92673](http://www.uniprot.org/uniprot/Q92673)), and Alamut visual ([www.interactive-biosoftware.com/alamut-visual/](http://www.interactive-biosoftware.com/alamut-visual/)).

We identified, among 24 other genetic variants (table e-1), rare variants in *SORL1* (NM.003105.5) and in the Teashirt Zinc Finger Homeobox 3 gene (*TSHZ3*, NM.020856.2). We used Sanger sequencing to confirm these rare variants, and to test other family members. The coverage of *SORL1* and *TSHZ3* captured with the exome kit was similar to the median read depth over the whole exome (table e-2).

We checked for occurrence of the variants in the exome aggregation consortium (ExAC) database ([exac.broadinstitute.org/variant/11-121416108-A-G](http://exac.broadinstitute.org/variant/11-121416108-A-G)), and in an aggregated cohort of 664 Dutch AD cases from the Amsterdam Dementia Cohort (ADC),<sup>13</sup> the Alzheimer center of the Erasmus medical center, Rotterdam, and the population based Rotterdam Study,<sup>17</sup> and in 1283 controls from the Rotterdam Study and from the 100-plus study on cognitively healthy centenarians ([www.100plus.nl/](http://www.100plus.nl/)).

### APOE genotyping

APOE genotyping was performed after genomic DNA isolation from 7-10 mL EDTA blood, using a QIAxcel DNA Fast Analysis kit (Qiagen, Venlo, The Netherlands).

## RESULTS

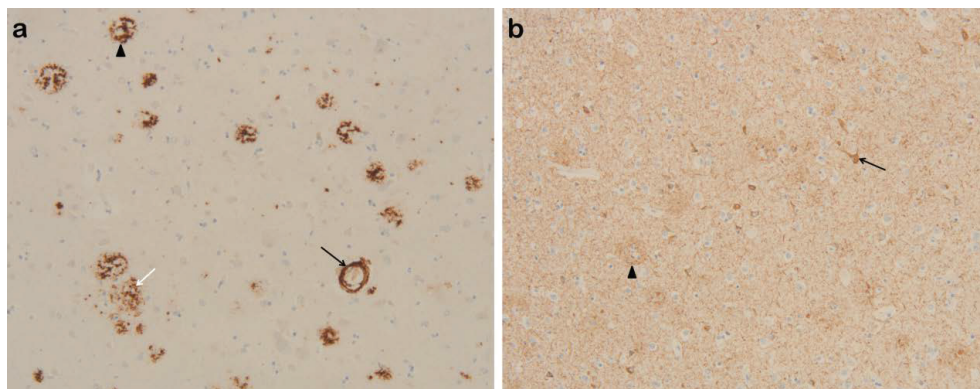
### Clinical description

Proband II.1 presented at the age of 67 with complaints of memory decline over the previous three years. The patient had suffered a skull fracture as a child. The mini-mental state examination (MMSE)<sup>18</sup> score was 25/30, and neuropsychological assessment showed impairment of episodic memory. Routine blood analysis was normal, except for increased serum cholesterol levels. Magnetic resonance imaging (MRI) showed mild bilateral hippocampal atrophy (medial temporal lobe atrophy (MTA) grade 1),<sup>19</sup> mild white matter hyperintensities (WMH), (Fazekas grade 1),<sup>20</sup> and no microbleeds. Electroencephalogram (EEG) revealed a discordant low background rhythm of six to seven Hz with increased amounts of intermitting delta activity in the frontotemporal regions. Cerebrospinal fluid (CSF) was not obtained. Based on these findings, the patient was diagnosed with mild cognitive impairment (MCI).<sup>21</sup> At the age of 69, MMSE was 21/30, repeated neuropsychological assessment showed progression of memory impairment and impaired executive functions. At this time, MRI showed biparietal atrophy, with no progression of the hippocampal atrophy or WMH. The clinical diagnosis was probable AD.<sup>22</sup> Disease progression was characterized by further deterioration in all cognitive domains, including the development of behavioral disturbances (loss of initiative and increased irritability). The patient was admitted into a nursing home, where the patient suffered from episodes of focal neurological deficits probably due to recurrent strokes and died at the age of 76 years.

Patient II.3 visited a geriatrician at a local hospital at the age of 72 because of memory complaints and fatigue for two years. The patient had diabetes mellitus type 2, hypertension and dyslipidemia. The patient had been treated for depression with amitriptyline for over 20 years. MMSE was 26/30 with disorientation in time, and neuropsychological testing showed impaired recall on memory tests. Computed tomography (CT) imaging showed mild diffuse cortical atrophy, aspecific hypodensities in the brainstem and in the basal ganglia. No formal diagnosis was made at that time. At the age of 74, the patient visited a neurologist for a second opinion. At this point, the patient reported the occurrence of headaches, and scored 23/30 on the MMSE. Routine blood analysis and EEG were normal. MRI and CSF analysis were not performed. The patient was diagnosed with probable AD. Diagnostic DNA testing revealed no variants in *PSEN1*, *PSEN2*, or *APP*. The patient died at the age of 78 years (cause unspecified).

Patient II.4 visited a local memory clinic at the age of 70 because of progressive memory complaints, which initiated at the age of 59 after a head trauma. The patient had suffered from bacterial meningitis at the age of 69. Neuropsychological testing showed impairments in concentration and memory, disorientation in place, and dyscalculia. No additional investigations were performed. The patient was diagnosed with probable AD. The patient died at the age of 74 years most likely due to a heart attack. Post mortem examination of the brain confirmed the diagnosis of severe AD (Braak stage 6/6 for tau and Thal phase 5/5 for amyloid) with extensive CAA type 1,<sup>23</sup> see figure 2.

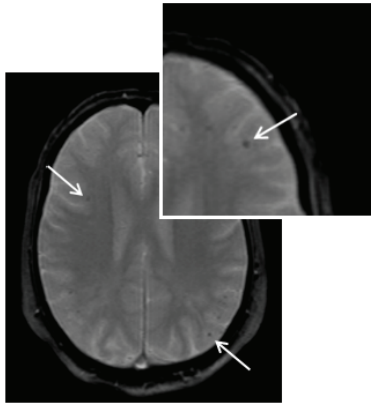
**Figure 2.** Neuropathology in subject II.4.



- a) Immunohistochemical training for abeta 1-17 on temporal cortex showing cerebral amyloid angiopathy (black arrow), classical plaques (arrow head) and diffuse plaques (white arrow)(10x obj.);  
 b) Immunohistochemical staining for tau (mab AT8) on temporal cortex, showing neuropil threads, (pre)tangles (arrow) and neuritic plaques (arrow head) (10x obj.).

Patient II.6 was evaluated at our memory clinic at the age of 70 years because of the positive family history of dementia. At this visit, the patient reported no cognitive complaints, MMSE was 30/30 and neuropsychological testing showed no abnormalities except for some difficulties with concentration. Routine blood analysis showed no abnormalities. MR imaging displayed no hippocampal atrophy (MTA grade 0), mild WMH (Fazekas grade 1), but a high number of 47 microbleeds, suggestive of CAA (figure 3). EEG showed a remarkably decreased background pattern with reactive alpha-theta activity till 8 Hz. CSF analysis showed a decreased amyloid-beta level of 232 ng/L (reference >550 ng/L), an increased total tau level of 993 ng/L (reference  $\leq 375$  ng/L), and an increased level of tau phosphorylated at threonine-181 (ptau) of 123 (reference  $\leq 52$  ng/L)). Based on the clinical examination, the patient was diagnosed with subjective cognitive decline (SCD).<sup>24</sup> During the following years, the patient developed memory complaints, loss of initiative and sleeping problems. At the age of 74 years, MMSE was 29/30, and neuropsychological testing showed disturbances in episodic memory. MRI showed no progression of WMH, but the number of microbleeds had increased to 58, and at reexamination at the age of 76 years, microbleeds had increased even further to 100. Repeated EEG displayed progressive slowing with theta activity of 7 Hz next to dominant posterior rhythms of 8 Hz. The patient was diagnosed with MCI, and referred to a local geriatrician for clinical follow-up. The patient was diagnosed with probable AD at the age of 82, with a MMSE score of 21 out of 30.

**Figure 3.** Baseline MR imaging of subject II.6



Cerebral MR imaging of subject II.6 at age 70 showing several microbleeds (arrows). T2 weighted image.

Patient III.1 presented at our memory clinic at 58 years with memory complaints, and self-reported difficulties with organizing and planning. The patient was treated for diabetes mellitus, hypertension and dyslipidemia. MMSE score was 29/30, performance



on neuropsychological testing was normal, and MRI showed no abnormalities. EEG was disturbed with a normal alpha background pattern of 9 Hz, but with early intermitting left predominant temporal theta activity. CSF concentrations showed a mildly decreased amyloid-beta level of 549 ng/L (reference >550 ng/L), increased tau level of 435 ng/L (reference  $\leq 375$  ng/L) and ptau level of 68 (reference  $\leq 52$  ng/L). Pittsburgh compound (PiB)-PET showed increased amyloid binding in all cortical areas. F18-fluorodeoxyglucose (FDG)-PET showed a normal pattern of glucose metabolism. Based on clinical evaluations, the diagnosis was SCD. The abnormal AD biomarkers indicated preclinical AD, with a high likelihood of underlying Alzheimer pathophysiology.<sup>25</sup> Over the next four years, the patient remained clinically stable.

Patient I.2 was reported to have had dementia with AD characteristics and to have died at the age of 85 years from a heart attack. No further information or DNA was available.

The oldest healthy (although not clinically investigated) individual of the youngest generation was included in the exome sequencing for segregation purposes.

DNA was also isolated from six other cognitive healthy relatives, all younger than 60 years of age.

**Table 1.** Results of genetic analysis

ID	Age at onset	Age at diagnosis	Age at death	Phenotype	<i>SORL1</i> Genotype	<i>TSHZ3</i> Genotype	<i>APOE</i> Genotype
II.1	65	69	76	AD	NA	NA	NA
II.3	70	74	78	AD	c.2021A>G	c.707C>T	$\epsilon 4/\epsilon 4$
II.4	61	70	74	AD (CAA)	c.2021A>G	WT	$\epsilon 4/\epsilon 4$
II.6	74	82	NA	AD (microbleeds)	c.2021A>G	c.707C>T	$\epsilon 4/\epsilon 4$
III.1	53	58	NA	preclinical AD	c.2021A>G	c.707C>T	$\epsilon 4/\epsilon 4$
III.2	NA	NA	NA	healthy	WT	c.707C>T	$\epsilon 4/\epsilon 4$
					c.2021A>G	WT	$\epsilon 4/\epsilon 4$
III.3-5	NA	NA	NA	healthy	c.2021A>G	WT	$\epsilon 3/\epsilon 3$
					WT	WT	$\epsilon 3/\epsilon 4$
					c.2021A>G	WT	$\epsilon 3/\epsilon 4$
					WT	WT	$\epsilon 3/\epsilon 4$
					c.2021A>G	WT	$\epsilon 3/\epsilon 4$

Abbreviations: NA= not available; AD= Alzheimer's disease; CAA= cerebral amyloid; angiopathy; WT= wild type

### Genetic outcome

We performed exome sequencing in two symptomatic siblings (II.3, II.6), and in two family members from the third generation, including one with preclinical AD (III.1), and one without cognitive complaints hitherto (III.2).

In *SORL1*, we detected the variant c.2021A>G:p.N647S in exon 14 in all three affected family members but not in the healthy family member (table 1). We did not detect this variant in a large cohort of 664 AD patients and 1283 controls, and the ExAC database reports just one heterozygous carrier of this variant. The variant locus is at a glycosylation site and highly conserved. The variant had a high CADD score of 22.2, was considered deleterious by PolyPhen and Mutation Taster, and segregated with disease in our AD family, indicating pathogenicity.

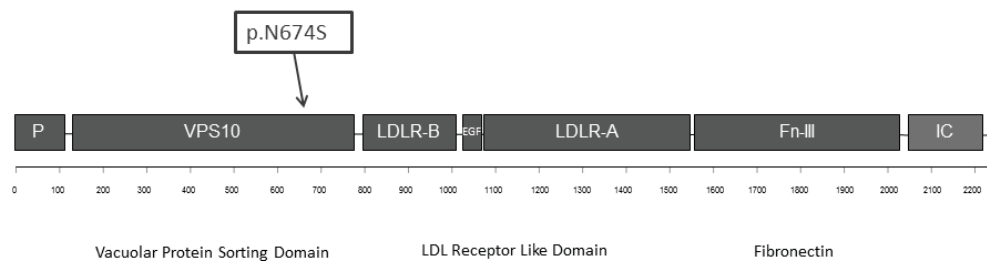
Another interesting variant was a missense variant in exon 2 of *TSHZ3*, c.707C>T:p.T236M. The *TSHZ3* protein has been reported to modulate APP trafficking and/or processing.<sup>26</sup> The p.T236M variant locus is highly conserved, and the variant had a CADD score of 20.7. However, the variant did not segregate with disease in our family (table 1), and was reported in 47 individuals in the ExAC database, suggesting low pathogenicity if any. All (preclinical) AD patients, and one healthy family member younger than 60 years had an *APOE* ε4/ε4 genotype.

### DISCUSSION

We describe a clinically heterogeneous AD family with no mutations in the common AD genes, in which we detected a rare variant in *SORL1*. The rareness and the high CADD score of the *SORL1* variant support a pathogenic effect. The product of *SORL1*, SorLA or LR11, belongs to the vacuolar protein sorting-10 receptor (VPS-10) family and to the low density lipoprotein receptor (LDLR) family (figure 4).<sup>27</sup> *SORL1* is widely expressed in the brain, predominantly in the neurons of the hippocampus. SorLA binds the major cholesterol-carrying lipoprotein of plasma LDL, including the ApoE rich lipoprotein β-VLDL, and transports it into cells by endocytosis.<sup>28</sup> Importantly, SorLA is involved in APP trafficking to and from the Golgi apparatus.<sup>27</sup> It acts as a sorting receptor that protects APP from trafficking to late endosome and from processing into amyloid-beta by preventing the cleavage of APP by alpha- or beta secretases. Moreover, newly synthesized amyloid-beta binds to the VPS-10 domain of SorLA which targets it to the lysosome for degradation.<sup>29</sup> Together, SorLA activity reduces the burden of amyloidogenic peptide formation, as suggested by a study with *sorl1* knockout mice.<sup>30</sup> The p.N674S variant is located in the VPS-10 domain and affects a N-glycosylation site, which is important for proper protein folding and for protein-protein interaction.<sup>27</sup> The previously detected *SORL1* variant G511R, also in the VPS-10 domain, was proven to disturb the interaction between SorLA and amyloid-beta, suggested to result in impaired lysosomal degradation.<sup>29</sup>

The *SORL1* variant occurred in combination with *APOE*- $\epsilon$ 4 homozygosity in all tested affected family members, which complicates the interpretation of the data as the homozygosity for *APOE*- $\epsilon$ 4 contributes to (preclinical) AD. *APOE*- $\epsilon$ 4 carrier status results in increased amyloid-beta production and less efficient clearance of brain amyloid-beta, thereby promoting amyloidogenesis and leading to an increased risk of developing AD compared to carriers of other *APOE* alleles.<sup>31</sup>

**Figure 4.** The protein product of Sortilin-related receptor gene (*SORL1*): SorLA



*SORL1* is located on chromosome 11q23.2-q 24.2 and codes for a 250-kDa membrane protein with seven distinct domains.

The rare variant is p.N674S.

The figure is based upon information from Uniprot (<http://www.uniprot.org/uniprot/Q92673>), transcript NM\_003105. P= Pro-peptide; VPS-10= vacuolar protein sorting domain 10; LDLR-B= LDL-receptor class B repeats; EGF= epidermal growth factor precursor type repeat; LDLR-A= LDL-receptor class A repeats; FN-III= fibronectin type-III repeats; IC= intracellular component; LDL= low density lipoprotein.

Experimental studies have shown that the two proteins encoded by *SORL1* and *APOE* may functionally interact. *APOE*- $\epsilon$ 4 has been hypothesized to reduce the capacity of SorLA binding with APP,<sup>32</sup> and a recent in vitro study showed that the uptake of extracellular ApoE4 was enhanced by overexpression of SorLA.<sup>33</sup> Furthermore, the uptake of extracellular amyloid-beta was *APOE*-isoform-dependent, with most uptake in presence of the *APOE*- $\epsilon$ 4 isoform. Hypothetically, a combination of a *SORL1* variant and the presence of *APOE*- $\epsilon$ 4 may therefore result in aberrant cellular amyloid-beta uptake resulting in increased amyloid plaque formation, while *SORL1* variants in *APOE*- $\epsilon$ 3 or *APOE*- $\epsilon$ 2 carriers may have no or less effect.

Interestingly, the phenotype in this family is heterogeneous. Whereas one patient showed extensive CAA at post mortem brain examination (II.4), and another (II.6) had multiple microbleeds on cerebral MRI suggestive of underlying CAA,<sup>34</sup> no microbleeds were seen on brain imaging of II.1 and III.1. Both *APOE*- $\epsilon$ 4 and *SORL1* are associated with an increased risk of microbleeds.<sup>35,36</sup>

Already during the preclinical phase of AD, one family member with and two without microbleeds showed increased theta activity and even intermitting delta activity on the

EEG. Such EEG patterns are typically detected in patients with moderate to severe dementia.<sup>37</sup> Previous studies described an effect of *APOE*-ε4 on EEG pattern, while the effect of *SORL1* loss of function on EEG patterns is unknown.<sup>38,39</sup> The prevalence of microbleeds/CAA and EEG abnormalities was not related to *APOE*-ε4 homozygosity and/or the *SORL1* variant in a consistent manner, suggesting the involvement of other genetic or non-genetic factors causing the heterogeneous clinical phenotype.

A limitation of our study is the absence of clinical data of some of the affected, and the young ages of the healthy family members, since preclinical AD cannot be ruled out.

So far, the evidence for the association between *SORL1* genetic risk variants and AD has been conflicting,<sup>10</sup> and to our knowledge only one study linked the presence of *SORL1* variants with familial AD with the suggestion of incomplete penetrance, although without reporting the *APOE* status in these families.<sup>12</sup>

Our data suggests that the homozygosity of *APOE*-ε4 in combination with the *SORL1* variant in the VPS-10 domain might underlie the pathophysiology of AD in this family. The variability in clinical features may be caused by modifying effects of additional (epigenetic) factors. Furthermore, a combined effect of more than one genetic factor, as in our family, may explain the difficulties to identify causal genetic variants in part of the assumed autosomal dominant AD families.

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## Supplementary material

Supplementary table 1. Genetic risk variants detected by whole exome sequencing.

Gene	Transcript	cDNA	protein	Chr <sup>a</sup>	Start position	Stop position	dbSNP <sup>b</sup>	OMIM	filter status <sup>c</sup>
<i>SERINC2</i>	001199039.1	c.973G>A	p.E325K	1	31905938	31905938		614549	PASS
<i>PUS10</i>	144709.2	c.874+2T>G		2	61188596	61188596	rs200065642		PASS
<i>FAM184B</i>	015688.1	c.1759A>G	p.K587E	4	17661646	17661646			PASS
<i>CCDC149</i>	173463.4	c.302G>A	p.R101Q	4	24854764	24854764			PASS
<i>ZNF479</i>	033273.1	c.257A>G	p.H86R	7	57193730	57193730	rs201001924		PASS
<i>GTF2IRD2</i>	173537.2	c.2146C>T	p.R716C	7	74211705	74211705	rs200699919		PASS
<i>DLID</i>	000108.3	c.803A>G	p.Q268R	7	107556069	107556069		238331	PASS
<i>AD4RB2</i>	018702.3	c.326A>C	p.H109P	10	1405974	1405974		602065	PASS
<i>RDX</i>	002906.3	c.-64-6delT		11	110150487	110150487	rs61003001	179410	PASS
<i>SORL1</i>	003105.5	c.2021A>G	p.N674S	11	121416108	121416108		602005	PASS
<i>ANO6</i>	001204803.1	c.1228+6delT		12	45771902	45771902	rs36059278	608663	PASS
<i>P4POLA</i>	001252007.1	c.179G>A	p.R60H	14	96986562	96986562		605553	PASS
<i>GOLGA85</i>	038843.1	c.1642-7_1642-5delCCC		15	23608818	23608820	rs30344467		PASS
<i>NECAB2</i>	019065.2	c.1078G>A	p.V360I	16	84035467	84035467			PASS
<i>MYH2</i>	001100112.1	c.4205G>A	p.R1402Q	17	10429176	10429176		160740	PASS
<i>DRG2</i>	001388.4	c.188C>T	p.A63V	17	17997250	17997250		602986	PASS
<i>SKOR2</i>	001278063.1	c.311G>A	p.R104H	18	44775244	44775244			PASS
<i>TSHZ3</i>	020856.2	c.707C>T	p.T236M	19	31769992	31769992		614119	PASS
<i>FUT1</i>	000148.3	c.551_552delAG	p.E184fs	19	49253987	49253988		211100	PASS
<i>IFNAR1</i>	000629.2	c.976A>G	p.T326A	21	34721584	34721584		107450	PASS
<i>COL6A2</i>	001849.3	c.2986G>C	p.V996L	21	47552392	47552392	rs142432514	120240	PASS
<i>CLTCL1</i>	007098.3	c.2529T>A	p.D843E	22	19209506	19209506		601273	PASS
<i>FAM104B</i>	001166703.1	c.175A>G	p.S59G	X	55172687	55172687	rs1047037		PASS
<i>FAM104B</i>	001166703.1	c.154A>C	p.I52L	X	55172708	55172708	rs1047034		PASS

a; chromosome

b; dbSNP build 138 (<http://www.ncbi.nlm.nih.gov/projects/SNP>)c; PASS if the variant complied with (i) GATK quality score  $\geq 50$ , (ii) quality over depth  $\geq 1.5$ , (iii) Strand bias  $\leq 60$ , (iv) total read depth  $\geq 5.0$ .Note, besides the variants detected in *SORL1* and *TSHZ3*, the variants are not confirmed by Sanger sequencing.

**Supplementary table 2.** Quality control and detailed information on *SORL1* variant p.N674S

Quality Control	
Genotype quality	99%
Filter <sup>a</sup>	PASS
Segregation	Confirmed in 4 affected family members
Sanger verification	YES
Chromosome	11
Position	g.121416108A>G (GRCh37)
Gene name	Sortilin-related receptor ( <i>SORL1</i> )
Mutation type	Missense
Transcript	NM_003105.5
cDNA-level nomenclature	c.2021A>G
Protein-level nomenclature	p.Asn674Ser
Population frequency <sup>b</sup>	1 allele in Latino population
Effect on splicing predicted	NO
Protein domain <sup>c</sup>	In glycosylation site
SIFT prediction <sup>d</sup>	Tolerated
Polyphen prediction <sup>e</sup>	Probably damaging
Mutation Taster prediction <sup>f</sup>	Disease causing
CADD <sup>g</sup>	22.2
Evolutionary conserved <sup>h</sup>	Highly conserved amino acid, up to Tetraodon (considering 11 species)

a; GATK Variant Quality Score Recalibration (VQSR) filter set at the default sensitivity threshold of 99%

b; Exome Aggregation Consortium (ExAC) database; <http://exac.broadinstitute.org/>

c; Uniprot database; <http://www.uniprot.org/>

d; SIFT; <http://sift.jcvi.org/>

e; PolyPhen2; <http://genetics.bwh.harvard.edu/pph2/>

f; Mutation Taster; <http://www.mutationtaster.org/>

g; CADD=Combined Annotation Dependent Deletion

h; Alamut Visual v.2.7.1; <http://www.interactive-biosoftware.com/alamut-visual/>